I appreciate the invitation to present some of our work and the Immunotherapy Program that we have here at Children's is really a team effort of the Brain Tumor Program at the Cancer Center as well as Pediatric Neurooncology who really does most of the work in managing the patients on this study and I'll acknowledge everybody in detail towards the end. Let me get started.

So the objectives of this talk are to review the current management and outcome for childhood gliomas and the rationale for considering using vaccines as a novel therapy for these tumors. I'll talk about some of the early preclinical work that was done in the lab that led us to this, some of our early study results and the challenges that we had with our initial vaccine approaches which were done predominantly in adults and then spend the rest of the time talking about the results in our Pediatric Glioma Vaccine Trial.

So the population group that's ideal for some sort of novel therapy are the ones that do particularly poorly with current therapies and one group of these are the diffuse brain stem gliomas. These are tumors that we see anywhere from 3 to 6 a year, and this is an example of one here. They present with rapidly progressive cranial neuropathies such as facial weakness or swallowing difficulties, long track signs such as hemiparesis or balance difficulties and they often have signal abnormality on MRI that extends from the front of the brain stem to the back of the brain stem. And the only modality, the only treatment that's shown any benefit for these tumors is radiation. Over the years I've been involved in numerous studies of novel chemotherapeutic agents, radiation dose intensification and none of them have been able to improve prognosis. And at present the one year

progression free survival is only about 15% and the overall survival is only about 35%. So these are probably about the worst kind of tumor you can have in either a child or an adult.

And these are some results, just illustrating that from the last – one of the last cooperative group studies that I was the Vice Chair on and this was a randomized comparison of two very intensive chemotherapy regimens followed by radiation therapy at extremely high doses. And you can see the survival curve here, the one year progression free survival for both of these regimens is less than 20%, so that's as good as it gets.

The non-brainstem malignant gliomas also have a poor prognosis and this is an example of one involving the thalamus. These tumors typically present with seizures or headaches, focal neurological symptoms such as hemiparesis and the only treatments that have shown benefit for these tumors are radiation therapy and alkylating chemotherapy agents like CCNU or more recently Temozolomide. There have been numerous studies of more intensive approaches that have all failed to improve prognosis and at present the one year progression free survival for these tumors is about 30%, so somewhat better and the overall survival is only about 50%. And these are results from the last cooperative group study for high-grade gliomas showing that in the favorable biological risk group the 2 year progression free survival was on the order of 25 to 30%, and in the unfavorable biological group that overexpressed MGNT, a drug-resistance gene, there were no survivors at 2 years. So those are two groups that are ideal for novel therapies.

Another group of gliomas that can be considered for novel therapies are the large midline low-grade gliomas. Now as neurosurgeons low-grade gliomas are a tumor type that we often can cure with surgery if they are on the surface of the cerebral hemispheres, if they are in the posterior fossa and the cerebellum we can take those out. But the ones that are deep in the midline of the brain like this one in a 2 year old we can't completely take out without hurting the patient. We often will debulk these, in some cases we'll make the diagnosis just based on MRI and these kids are treated with chemotherapy, usually that will hold the tumor in check for several years. This particular patient had a very nice response to chemotherapy but then the tumor began to grow back, she got additional chemotherapy, ultimately wound up getting radiation therapy and then more chemotherapy down the road. So these tumors will often progress multiple times and not be cured. So even for this type of tumor, a benign low-grade glioma, there is a need for new therapies. And this just illustrates some unpublished data from the cooperative group trial recently completed showing that these midline tumors if you follow them long enough have about an 80% chance of progressing despite chemotherapy.

So over the years our Brain Tumor Program which has been funded by a large program project grant from the NIH has focused on looking at novel therapies for tumors both in kids and adults. And one of the areas that we focused on is immunotherapy. It would be very appealing if you could identify antigens in the tumor and vaccinate the patient the same way you would for any of the normal childhood viruses. So our goal was to try to develop an effective immunological approach for malignant gliomas in kids and adults and our hypothesis was that we could induce specific anti-tumor immunity either by transducing the tumor cell with a virus to make it express a cytokine that

would rev up the immune system, or by identifying antigens in the tumor that we could use as a vaccine cocktail. And over the years we've experimented with both of these approaches. We started out doing some laboratory studies on cytokine gene transduced glioma vaccines and then launched an FDA approved clinical trial. I'll tell you about those results. There were some limitations with that approach particularly for pediatric tumors, so we began screening for tumor antigens and ultimately identified a number that we have since moved back into clinical trials both for adults and kids.

So our initial approach as I mentioned was a gene transduced vaccine, and what that involves is taking a glioma cell, a brain tumor cell and transducing it with a gene that will produce a cytokine like Interleukin 2 or Interleukin 4 or GM CSF that revs up the immune system and makes the tumor cell recognized by the immune system and then reject it. And we tested a whole laundry list of these cytokines in mouse models. This is supposed to be a mouse. And of those cytokines the one that was the most effective in several of our models was Interleukin 4. And this was a couple of years of work to prioritize that as the cytokine to move forward in clinical trials. We filed an investigational drug application with the FDA and got approval to do a IL 4 transduced glioma vaccine trial in adults with recurrent resectable high-grade gliomas.

In order to get this through the FDA we had to add two things to the IL 4, a Neomycin gene so that we could select the transduced cells in culture and also find it in kinase so that we could kill off the vaccine cells with Ganciclovir. Ganciclovir is converted by thymidine kinase into a toxic metabolite and any cell that has the TK will be killed and the normal cells won't.

So the approach that we took was, and at this point I was doing some adult brain tumor surgery. We would resect the tumor and then grow it in culture for anywhere from 4 to 8 weeks. Once we had enough cells we would transduce those with the viral vector and then expand that and then immunize the patient with the transduced cells. Then after 2 weeks we would give Ganciclovir to wipe out the vaccine cells and then we'd repeat the process over again. And this is just an example of one patient's arm showing that we used different doses of this vaccine and the patient actually had a local immune response to the vaccine, which is what you would expect if the vaccine was having an effect.

We also biopsied some of those vaccine sites, this is an example of one vaccine site 14 days after the vaccination, these little black dots are lymphocytes, so this area is filled with lymphocytes reacting against the tumor and the – and the cytokine. A control area nearby had no response and then we did a secondary vaccine and confirmed at baseline there was no inflammation but after a week in this case there was significant inflammation. One of the things the FDA required was that we demonstrated that we could eliminate the transduced vaccine cells, so we did RT PCR for the neomycin gene and demonstrated that neomycin expression was seen early on at day 1, day 2, by day 7 and day 14 it was gone. So the thymidine kinase was killing off the vaccine cells.

This study ran in the early 2000s and we had some really dramatic results. This is a 63-year old fellow who had a four time recurrent glioblastoma. This is his pre-vaccine and 6 month post-vaccine MRI showing a pretty dramatic regression of the tumor and the mass effect from the tumor is also

diminished. And this is the same scan but a T2 weighted image showing that all the mass effect that was here pretreatment is gone post-treatment. We had a number of responses like this. This is another patient, doesn't project well with the light but had enhancing tumor here pre-vaccine and by 9 months post-vaccine it's all gone. So this patient had a complete response. And for an adult glioblastoma which is about as bad a diagnosis as a pediatric brainstem glioma, to have a complete response to anything is pretty unusual.

So we were very excited about this but there was one problem with this approach. These tumors grow pretty rapidly and if you remember I mentioned it takes four to eight weeks to grow the vaccine. During that time we had a lot of patients that signed up for the vaccine study but by the time they were ready to get vaccinated their tumor had grown back, it was big, they needed to get re-resected, needed to get other therapies. So from a practical standpoint this was not a winner. Even though we got good responses, it wasn't applicable to the majority of patients that had malignant gliomas. And it definitely wasn't applicable to deep seated tumors where you couldn't get a biopsy sample or to pediatric brainstem gliomas which we never biopsy. So we needed something else.

And the approach that we took was to try to identify antigens in the tumor that we could use as a antigen-based rather than a whole cell vaccine approach. There are a number of strategies to identify tumor antigens. One approach that we took early on was something called SEREX which is a serological examination of recombinant DNA expression libraries. That's why they call it SEREX because that's way too much to say. But what it involved is taking mRNA from the tumor tissue, putting it in a bacteriophage library and then plating it out on a bacterial lawn. This is then overlaid

with nitrocellulose and blotted with serum from patients that responded to the tumor. So we took the positive responders from the initial trial and then had them, had their serum screened against their own tumors to try to figure out what they were reacting against. We came up with a lot of interesting antigens. The problem was that most of them had characteristics that made them good for recognition by the humeral immune system but not by the cellular immune system. And the other problem was that many antigens were also expressed on normal cells so they were not ideal targets for a peptide vaccine.

So we then took a different approach which was to look at DNA expression data that's pretty widely published in the literature. To look at proteins that overexpressed in gliomas and not in normal tissue and use those. So this was sort of coming up with a prioritized list of candidates. One of the ones that we selected, something called IL13Ra2, it's overexpressed in the majority of malignant gliomas for reasons that nobody knows. But it's not expressed in normal cells with the exception of the testis. So it is a cancer testis type antigen which is very similar to the expression profile that's seen in a lot of the antigens that are used in adult melanoma vaccines and renal cell carcinoma vaccines. So we thought this would be one good candidate. We used a variety of algorithms to identify HLA binding sites and the HLA system is what helps T cells recognize foreign antigens in the body. We specifically looked at HLA-A2 binding sites because A2 is the most common HLA phenotype in North America. So we figured if we could identify these we would come up with a series of peptides that might be applicable for about half of patients.

And it turns out that IL13Ra2 was over expressed in most malignant gliomas. This is a selection of glioma cell lines just to illustrate that. These two are HLA-A2 positive and express IL13Ra2. This one is HLA-A2 positive but doesn't express IL132Ra2 and this one is IL13Ra2 positive but it's HLA-A2 negative. So what you might expect if we raised a cytotoxic T cell line against IL13Ra2 and it was an HLA-A2 background, it would kill these cell lines but not either of these cell lines and we did that experiment and we did a chromium release assay using a cytotoxic T cell line and it specifically lysed the two glioma cell lines that we expected it would as shown here, it didn't lyse the cell line that didn't express IL13Ra2 and it didn't lyse the cell line that expressed IL13Ra2 but was HLA-A2 negative. This may be way too much M8C stuff to talk about but it actually was important for us to confirm that if we picked peptides out from antigens that we were identifying that we could kill tumor cells that express that antigen and had the right HLA phenotype.

We went through this process with a whole bunch of antigens and ultimately came up with a cocktail that had four of the antigens that were most highly expressed in adult malignant gliomas and we put that together in a clinical trial for adults. We also added an immunoadjuvant to the peptides, polyICLC which is a ligand for the toll-like receptor 3 that we had done some preclinical work to suggest that this would enhance the effect of vaccination.

So in the adult trial which I'll talk about for a couple minutes, the main objective was safety. To make sure that we weren't causing brain melt by putting, by vaccinating the patient and causing a reaction against normal astrocytes or neurons or other things. And our secondary objective was to see whether we were having a response either immunological or clinical and our immunological

response was assessed by something called ELISPOT which is a functional measure of lymphocyte activity and tetramer assays which is a quantitative measure of lymphocyte numbers.

The adult trial enrolled 22 patients and the vaccine approach was well tolerated. We had no abnormal brain reactions, there were no grade 3 or 4 adverse events of any sort. The most common toxicities were just related to the polyICLC adjuvant, fever, chills, headache, sort of flu-like symptoms. But other than that it was quite acceptable. We also did a detailed immunological characterization and I apologize for the complexity of this slide but what I want to focus on is this column which are the ELISPOT results. And the positive results are shown as red, yellow or brown. The negative results are in green and these are ELISPOT results to a variety of different antigens within the peptide cocktail. So if you look at this, more than half of the tumors, more than half of the patients I should say responded to at least one of these tumor antigens. Overall, 11 of 19 responded to the initial series of vaccinations and then these patients also got boosters and a couple more responded to the boosters.

And there were some fairly dramatic clinical responses as well. This looks great on the screen, it doesn't look quite as good up there but this is a patient with a posterior temporal tumor pretreatment and 9 weeks posttreatment showing regression of the tumor. We also had some patients that had tumors that were stable over a prolonged period of time. One of these patients got biopsy and the biopsy site interestingly didn't show any residual tumor, this is a picture of the biopsy site but what it did show was an infiltration of lymphocytes and macrophages. So these are CD68 positive

microphages, these are CD8 positive lymphocytes showing that there's significant infiltration of immune cells into the site of the previous tumor.

And finally we had one patient that had a complete response and this patient, here's the tumor pretreatment, 9 weeks post-vaccine showing that it's getting smaller and then by 17 weeks it's gone and this patient had a prolonged complete response. So again another adult malignant glioma particularly bad type of tumor that had long term survival and regression of his entire tumor. Overall, 16 of 22 patients are alive more than a year after the vaccine and 6 of them are alive more than 2 years, so a fairly good result for a fairly difficult population of tumors.

There was a correlation between immune response and clinical response which is what one would expect if the vaccine was actually having an effect on the immune system. Patients that had positive tetramer reactivity or ELISPOT reactivity did significantly better than those that didn't.

So in summary for the adult part of this, the regimen was well tolerated, immune responses were seen in just over half of the patients and a lot of these patients were long term progression free survivors. Nine of them are progression free more than 12 months and 1 had a sustained complete response.

In addition the clinical responses correlated with immune responses. So it means that the vaccine is actually doing something productive and that is translating into an improvement in outcome. So based on this adult data we began asking ourselves could we do the same thing in our pediatric

patients. We were fortunate to get NIH funding to do a pediatric trial of this vaccine strategy, we also got foundation funding which we were quite grateful for. And as a first step in doing this we wanted to see which peptide antigens to include in the vaccine. So we screened a whole bunch of tumors in kids for a number of these antigens. And ones that were expressed most consistently in the tumors were EphA2 which is a ligand for the ephrin receptor, IL13Ra2 which is one of the antigens we used in the adult trial and survivin which is overexpressed in most forms of cancer. So these were three logical targets and they were all overexpressed.

Overall 13 of 15 brainstem gliomas and all 12 non-brainstem gliomas expressed at least one of these antigens and many of the tumors expressed two and some even three of these antigens. We also while we were doing our results there was a group from NYU that was doing a similar study using RT-PCR, we used immunohistochemistry but they came up with essentially identical results that these group of antigens was actually a pretty good choice to include in a vaccine.

So we concluded that the approach was reasonable and we then moved this forward into a clinical trial and we have an IND for this study just like the adult study, the primary objective as with the adult study is to demonstrate that this safe and reasonably well tolerated and secondarily to look at immune response in these patients.

The antigens that we've included include IL13Ra2, EphA2 and survivin, ones that I mentioned a few moments ago. We also added Tetanus toxoid which is a T helper peptide and most people have been exposed to Tetanus toxoid so it's a logical target to use as a way of boosting the immune system.

The vaccine is administered subcutaneously every 8 weeks and it's emulsified in a mineral oil base called Montanide and administered with intermuscular polyICLC the toll-like receptor ligand.

So the study includes several strata, we have a strata for newly diagnosed diffuse intrinsic brainstem gliomas treated with radiation alone or with radiation and chemotherapy. We have a strata for newly diagnosed non-brainstem malignant gliomas treated with radiation alone or radiation plus chemotherapy and then we have strata for recurrent low grade and high grade gliomas.

This lists our eligibility criteria. The three relevant ones that I want to mention are that the patients have to all be HLA-A2 positive by flow cytometry because the vaccine only works in patients that are HLA-A2 positive as far as we know. They can't have received chemotherapy after radiation because chemotherapy will suppress the immune response. And they have to be either off corticosteroids or on very low doses of corticosteroids for at least a week prior to registration because corticosteroids will suppress the immune response.

The study is designed to have 6 subjects in each stratum but if safety is demonstrated in the first 6 then we can enroll an additional 6 to get additional information regarding safety and immunological activity. So the first step in the enrollment process for people who are applying for this study, is HLA typing to make sure that they're A2 positive. This can be done anytime after diagnosis for the newly diagnosed patients and anytime after recurrence for the recurrent patients. The patients that are HLA-A2 positive with recurrent disease can go directly on the vaccine. The newly diagnosed

patients have to complete radiation and then an interval after radiation to let them get over the side effects of radiation and then they can go on vaccine therapy.

This is a schema that just shows what happens on this study. For the newly diagnosed patients either with brainstem glioma which are usually diagnosed by imaging or high grade gliomas which are diagnosed based on biopsy, the patients will get radiation therapy as a first step because radiation is a part of standard therapy. We don't want to eliminate standard therapy, we want to build on it. Then when they complete radiation and after an interval they get baseline MRI evaluations and then begin vaccination with this peptide cocktail and polyICLC every 3 weeks for a total of 8, and then we get MRIs and peripheral blood mononuclear cells for immune response analysis at 3 time points.

And for the recurrent patients it's exactly the same, except that the baseline studies are done at enrollment, they don't have to get radiation since they've already had it in most cases and then they go on and start the vaccination regimen. The patients also have the option of getting continuation therapy, so if they are doing well they can keep getting vaccines at a less frequent interval after the first 8.

The laboratory studies that we do as part of this protocol include the ELISPOT assay which I mentioned earlier is a functional assay of lymphocyte responses, the two lymphocytes produce interferon gamma which is indication that they are revved up to the tumor antigen. The other analysis is tetramer which tells what percentage of lymphocytes in the blood stream are reacting against a given antigen. For IL3Ra2 for example normally if you drew blood from one of us there

would be almost no cells that would react against this. But after the vaccination we hope to see that the percentage of cells would go up. And we're also evaluating the tumor tissues themselves for antigen expression.

So I may be slightly off on the enrollment numbers, this was my summary from a couple of weeks ago, but I believe about 106 patients have been screened on this study. And when you consider that there are only about 200 new brain stem glioma patients and high grade glioma patients in North America every year, the fact that in 18 months or so we've screened 106 gives you an idea of how widely people are referring patients here to potentially be enrolled on this study. So there's a lot of interest in this study.

About 40 have been HLA-A2+, we've found that a lot of the low grade gliomas that we've screened for whatever reason are HLA-A2-, more of the high grade gliomas are HLA-A2+. And we've enrolled 20 patients, 9 newly diagnosed brain stem gliomas that have been treated with radiation alone, one newly diagnosed patient with brain stem glioma who got chemotherapy and radiation, 5 newly diagnosed high grade glioma patients and 5 recurrent patients, and enrollment on the study continues.

The regimen in general has been well tolerated, this just summarizes the toxicity after course 1, the main toxicities as in the adult trial are flu-like symptoms or injection site reactions, so kids are sore at the site of the vaccine, or they get flu-like symptoms from the polyICLC. In general this has been well tolerated, the only significant adverse events that we've seen are 3 patients have had what

we've described as pseudo tumor progression, their tumor actually gets bigger and then stabilizes on corticosteroids and in some cases gets smaller again. And they have worsening neurological symptoms during this time. So we've worked out a very elaborate protocol for managing kids that have potential pseudo progression and I'll show you an example of that in a moment.

I apologize for this complicated summary slide but since this study is a work in progress we keep updating our results summary. The positive results are shown here as shaded boxes. Of the 18 patients that have been followed long enough to look at response analysis 16 of them have made it through their first 2 vaccination cycles, which is encouraging. One of them has had a prolonged absence of disease after a complete resection, 2 others have had partial tumor regression, one of which quite dramatically. And these boxes under outcome show patients that have already exceeded the median survival expected for their particular tumor type, so at this point about70% of the patients that have been evaluated long enough have beaten the median survival for brain stem gliomas for example. Now that's not a statistically significant number of patients, but it gives us some hope that this vaccine is actually having some activity. And in terms of the immunological analyses 4 of 5 patients that we've analyzed so far have had positive immune responses to the vaccine.

On ELISPOT we've had positive responses to IL13Ra2, in 4 cases to EphA2 in 2 cases and to survivin in one, we've had positive tetramer responses and one other interesting point is that all 4 tumors that we have been able to get tumor samples on have expressed at least one vaccine antigen. So it fits with our, our preliminary data that these antigens are widely expressed.

I included this slide just to show the complexity of matching the patient's immune response to the phenotype of the tumor. If you induce a big immune response to a particular antigen and the tumor doesn't express it, it's not going to do anything. In this particular patient there was a very strong immune response to IL13Ra2 shown here. Unfortunately on immuno-histochemistry the tumor didn't express IL13Ra2. There was a moderate response to EphA2 shown here, unfortunately this tumor expressed EphaA2 in a very patchy distribution. So you would not have predicted that this patient would have had a particularly good response clinically, and although we did have an immunological response the clinical response was disappointing.

The same thing with the tetramer assay, this is a patient that had a positive tetramer response to EphA2, at baseline there are almost no tumor, or no mononuclear cells expressing or reacting against EphA2. After the vaccination the number goes up to 1%, which is a pretty large percent when you consider that 1% of the cells in this patient's body were reacting against this vaccine antigen. But the tumor didn't express it, so the tumor progressed and the patient was started on corticosteroids and the immune response went down. So there has to be a matching between the antigen that the patient is reacting against and the antigen profile in the tumor. And even using three peptides you don't always have a hit.

I want to show a couple examples of positive responses that we've had. This is a child that had pseudo tumor progression. This is a 10 year old girl, and I'm sorry that it looks a little bit dark, who had a diffuse brain stem glioma, presented with rapidly progressive cranial nerve deficits and long tract findings. You can see it here, here the tumor is this area. It will show up better on the next

slide. This child was treated with radiation. Here you can see the tumor, it basically fills the entire brain stem, there is no normal brain stem here, it's all engulfed by tumor. So after radiation therapy this child went on to vaccine therapy.

And in December after 4 months of vaccination the patient began getting worse. We figured the child was progressing, these tumors have a median progression free survival of 5 to 6 months, so we figured the tumor was progressing and after progression these tumors do terribly, so the child was placed in hospice care and started on corticosteroids. So have this picture in your mind of how bad this looks, the whole brain stem is really swollen and then a month later on corticosteroids all of a sudden the patient was doing better and the tumor has dramatically shrunk on both T2 and T1 weighted images. So we thought this could be a steroid effect but not likely. So we repeated a scan 2 months later on much lower doses of steroids, and the tumor is essentially gone, there are – there are some signal abnormalities in the area where the tumor was but there is basically a hole in that part of the brain where the tumor had previously existed. And this is a scan from August, so this child is almost 17 months from diagnosis and a tumor type where the median survival is on the order of 8 or 9 months. And this child is alive at this point with no evidence of progressive tumor.

This child had a very prominent immunological response on ELISPOT assay. Six weeks after starting the vaccine there was a spike in ELISPOT response to IL13Ra2. It remained high. This is when the pseudo tumor progression was documented, steroids were started and the response went down, which is what you would expect. The steroids were weaned around here and the response started going back up.

One other positive response, this is a 16 year old with a thalamic glioblastoma, pretreatment – pretreatment, this is post radiation therapy here, this is after two vaccines, 5-7-11 the tumor has substantially shrunk over time. Again for tumors like this, this is pretty unusual and we are pretty encouraged by this.

So in conclusion, our vaccine based approach has been well tolerated in children with gliomas. We've seen immunological and clinical evidence of activity and this is encouraging but clearly more extensive analyses of efficacy are needed to see whether this should move up in terms of the treatment hierarchy for these tumors. And we have put together a Phase II multi-institutional study for the Pediatric Brain Tumor Consortium which will involve this vaccine approach with slight modifications for newly diagnosed brain stem gliomas and recurrent high grade gliomas. And this will be the first time that a vaccine approach has been translated in a multi-institutional context for pediatric brain tumors.

I'd just like to take the time to acknowledge all of the people that have been involved in this. It's been a very large effort. On the pediatric end the screening and management of these patients is really done by the Neurooncology Group led by Reggie Jakacki, and she has a great team of people, Angie Krol, Heidi Haynes, Jennifer Paoletti and Sharon Dibridge, who are involved in the patient screening, all the regulatory things that go into getting a trial like this approved and make sure that our data analysis is staying current. It's a huge amount of effort.

For the immunological monitoring, that's done in the Immunological Monitoring Laboratory at the UPCI led by Lisa Butterfield. We are fortunate to have a resource like that here at Pitt that we can call on. Hideho Okada works with me in the Brain Tumor Program, co-directs the program with me and has done a lot of the preclinical work and also the adult trials. And then Ron Hamilton has done a lot of the path analysis. And then there is clearly as you can see a whole fleet of other people that have been involved in various contexts with this, so it's been a big team effort and things have progressed along well over the last several years.

I'd be happy to take any questions.

Very exciting data, I'm wondering if you have any data on the antigen specificity of the lymphocytes in the tumor biopsy, whether they would correlate to the same ELISPOT positive, tetramer positive cells that you've found in the circulation?

We haven't done the – we haven't done that on these since we have to this point only gotten biopsies, post-treatment biopsy tissue on a couple patients and they've been very small amounts of tissue. So we've mainly been looking at percentage, or numbers of lymphocytes and things like that that are there but haven't gotten enough to look at tumor infiltrating lymphocytes qualitatively to see what they are reacting against. That would be a very important thing to do if we had larger amounts of tissue. Yeah?

Okay, this is really exciting. Two questions, a simple one, by the strategy you give the vaccine IM rather than sub-Q?

So the vaccine is sub-Q, the polyICLC, the adjuvant we give IM.

And will it work without the adjuvant?

Well that's a good question.

You said the clinical studies but –

Yeah, that's a good question. It's something that we are wrestling with on the Phase II study because most of the toxicity comes from the polyICLC and we are thinking about other things – using other things like GM CSF for example. In our preclinical models we did have good results with the peptides alone, but the results with an adjuvant were even better, which is why we you know moved it forward that way.

And the second question is are the peptides you use unique to the tumor tissue, or are they expressed normally in any other tissues of the body?

The IL13Ra2 is only expressed on tumor and in testes, so it's one of those cancer testes antigen types, it's very restricted in its expression. The – I guess the Ra2 is a variant that's not normally

expressed in the body, so it's not expressed on normal lymphocytes for example. Survivin is expressed more in tumor cells than in normal cells but it also is expressed in stem cells. We haven't seen any toxicity in terms of hemologic or you know anything like that which you might expect with targeting survivin. But yeah, and EphA2 is expressed in some other cells as well, so they are not 100% specific.

Have you looked at effect on say bone marrow or any other cells?

Well yeah we follow blood counts and you know hemologic parameters during the course of the vaccine and we haven't seen any, any drop-offs.

It's really nice, Ian. I was just wondering is there any correlation between – I don't know if I was a parent of a child that was receiving this vaccine, I think I'd be real excited if they had a severe local response to the vaccine, indicating that their body was responding to it. Have you noticed any correlation between you know the type of reactions occurring locally and the response to the tumor itself?

Reggie, do you want to comment? You may have a better handle on that than me.

We have seen some huge local reactions _____, even though it's painless _____. Some of the mitigating factors – just to repeat that, we have seen some very impressive local reactions. We had one girl with the site actually started to sluff a little bit although it was painless. One of the

mitigating factors is that a lot of these kids come from very far away and so then they hop onto a plane to go back home or they sit in a car for 6 hours to go back home, so it doesn't circulate as much and that may impact how much of a local reaction they have. We also don't have all of the biologic correlative results back yet to correlate that with the reactions, but we are keeping track of how big the induration in the local sites are, so that's something that we'll evaluate more thoroughly once we have all the data.

Okay, thank you.